

Supplementary Material

Supplementary material to the paper ‘Development of an IntelliCage based Cognitive Bias Test for Mice.’

Table S1: Experimental schedule of group two. Schedule of the presentation of the tone-frequencies. For each IC corner visit one tone-frequency was randomly played. After hearing the positive tone-frequency, the mice had to perform a nosepoke to receive water. After hearing the negative tone-frequency, the mice must not perform a nosepoke otherwise they receive an airpuff.

day	Tone/Treatment	Tone length
-6 - 0	Visit open doors	No tones
1 - 6	positive tone	0.5 sec.
7 - 13	5% probability for negative tone	0.5 sec.
14 - 20	10% probability for negative tone	0.5 sec.
21 - 27	10% probability for negative tone	1.0 sec.
28 - 34	16% probability for negative tone	1.0 sec.
35 - 41	20% probability for negative tone	1.0 sec.
42 - 48	33% probability for negative tone	1.0 sec.
49 - 84	Break, out of set-up	No tones
85 - 98	Visit open doors	No tones
99 - 100	positive tone	1.0 sec.
101 - 102	10% probability for negative tone	1.0 sec.
103 - 104	20% probability for negative tone	1.0 sec.
105 - 111	50% probability for negative tone	1.0 sec.

Table S2: Experimental schedule of group three. For each IC corner visit one tone-frequency was randomly played. After hearing the positive tone-frequency, the mice had to perform a nosepoke to receive water. After hearing the negative tone-frequency, the mice must not perform a nosepoke otherwise they receive an airpuff. In addition, during cognitive bias test three ambiguous tones-frequencies were presented, a nosepoke had no consequences.

Day	Tone-Frequencies/Treatment	Tone length
-4 - 0	Nosepoke open doors	No tones
1	positive tone	2 sec.
2 - 4	20% probability for negative tone	2 sec.
5 - 7	All doors open	No tones
8	20% probability for negative tone	2 sec.
9 - 11	50% probability for negative tone	2 sec.
12 - 14	All doors open	No tones
15	20% probability for negative tone	2 sec.
16 - 18	50% probability for negative tone	2 sec.
19 - 21	All doors open	No tones
22	50% probability for negative tone	2 sec.

23 - 25	baseline 1	2 sec.
26 - 28	All doors open	No tones
29	50% probability for negative tone; negative housing conditions and restrain test	2 sec.
30 - 32	Cognitive bias test 1; negative housing conditions and restrain test	2 sec.
33 - 35	All doors open, negative housing conditions	No tones
36	50% probability for negative tone; negative housing conditions and restrain test	2 sec.
37 - 39	Cognitive bias test 1; negative housing conditions and restrain test	2 sec.
40 - 42	All doors open, normal housing conditions	No tones
43	50% probability for negative tone	2 sec.
44 - 46	Cognitive bias test 1 baseline 2	2 sec.
47 - 56	All doors open	No tones
57	20% probability for negative tone	2 sec.
58 - 60	50% probability for negative tone	2 sec.
61 - 63	All doors open	No tones
64 - 67	50% probability for negative tone	2 sec.
68 - 70	All doors open	No tones
71	50% probability for negative tone	2 sec.
72 - 74	Cognitive bias test 2 baseline 1	2 sec.
75 - 77	All doors open	No tones
78	50% probability for negative tone	2 sec.
79 - 81	Cognitive bias test 2 baseline 2	2 sec.
82 - 83	All doors open	No tones
84	50% probability for negative tone; negative housing conditions and restrain test	2 sec.
85 - 88	Cognitive bias test 2 negative housing conditions and restrain test	2 sec.
89 - 94	All doors open, normal housing conditions	No tones
95	50% probability for negative tone	2 sec.
96 - 98	Cognitive bias test 2 baseline 3	2 sec.

Table S3: Results of the comparison of the performed nose pokes in response to the positive and negative tone-frequencies in group two. The data for experimental day 23, 99, 104, 105 is missing due to technical problems with the IntelliCage system. No data from day 49 to 84 is available, because the mice were not in the home-cage based set-up. No tones were presented between experimental days 85 and 98.

Day	Estimate	SE	DE	t.Ratio	p-Value	Treatment	Tone length
7	-2,64372	8,35	452	-0,317	1	5% neg. tone	0.5 sec.

8	2,78786	7,81	452	0,357	1	5% neg. tone	0.5 sec.
9	-9,39905	7,43	452	-1,265	1	5% neg. tone	0.5 sec.
10	-4,45765	8,71	452	-0,512	1	5% neg. tone	0.5 sec.
11	3,73997	7,81	452	0,479	1	5% neg. tone	0.5 sec.
12	-0,93763	8,05	452	-0,116	1	5% neg. tone	0.5 sec.
13	11,45462	7,6	452	1,507	1	5% neg. tone	0.5 sec.
14	12,11881	7,6	452	1,594	1	10% neg. tone	0.5 sec.
15	-9,22274	7,43	452	-1,242	1	10% neg. tone	0.5 sec.
16	5,20748	7,6	452	0,685	1	10% neg. tone	0.5 sec.
17	13,29123	7,6	452	1,748	1	10% neg. tone	0.5 sec.
18	-0,58871	7,6	452	-0,077	1	10% neg. tone	0.5 sec.
19	9,63357	7,96	452	1,21	1	10% neg. tone	0.5 sec.
20	2,42426	7,76	452	0,312	1	10% neg. tone	0.5 sec.
21	-41,36665	7,96	452	-5,198	0,0012	10% neg. tone	1 sec.

22	-47,77486	7,96	452	-6,003	<.0001	10% neg. tone	1 sec.
23	No data					10% neg. tone	1 sec.
24	-33,38427	8,2	452	-4,073	0,1162	10% neg. tone	1 sec.
25	-47,49622	7,76	452	-6,122	<.0001	10% neg. tone	1 sec.
26	-55,35421	7,96	452	-6,955	<.0001	10% neg. tone	1 sec.
27	-35,25731	7,96	452	-4,43	0,0332	10% neg. tone	1 sec.
28	-42,02188	7,76	452	-5,417	0,0004	16% neg. tone	1 sec.
29	-49,67321	7,76	452	-6,403	<.0001	16% neg. tone	1 sec.
30	-66,7605	7,76	452	-8,606	<.0001	16% neg. tone	1 sec.
31	-44,7429	7,76	452	-5,767	0,0001	16% neg. tone	1 sec.
32	-53,58103	7,96	452	-6,733	<.0001	16% neg. tone	1 sec.
33	No data					16% neg. tone	1 sec.
34	-43,41529	7,76	452	-5,596	0,0002	16% neg. tone	1 sec.
35	-60,3052	7,76	452	-7,773	<.0001	20% neg. tone	1 sec.

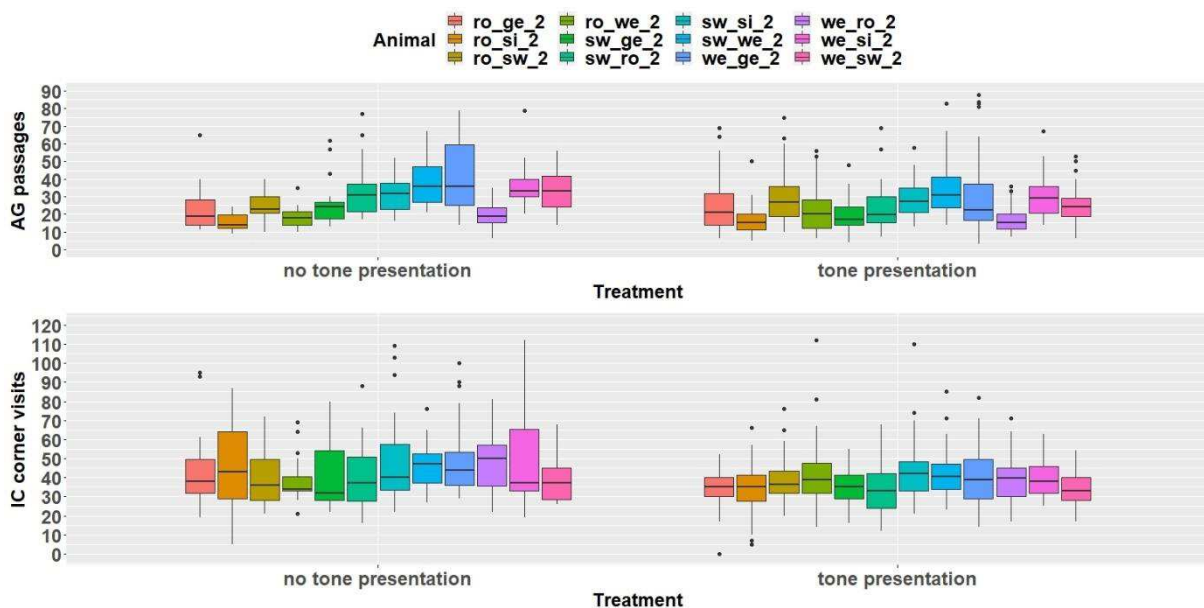
36	-44,44735	7,76	452	-5,729	0,0001	20% neg. tone	1 sec.
37	-57,09849	7,76	452	-7,36	<.0001	20% neg. tone	1 sec.
38	-60,80216	7,76	452	-7,837	<.0001	20% neg. tone	1 sec.
39	-53,69022	7,76	452	-6,921	<.0001	20% neg. tone	1 sec.
40	-50,05107	7,76	452	-6,452	<.0001	20% neg. tone	1 sec.
41	-50,39234	7,76	452	-6,496	<.0001	20% neg. tone	1 sec.
42	-54,16668	7,76	452	-6,982	<.0001	33% neg. tone	1 sec.
43	-52,43819	7,76	452	-6,759	<.0001	33% neg. tone	1 sec.
44	-50,07023	7,76	452	-6,454	<.0001	33% neg. tone	1 sec.
45	-50,13788	7,76	452	-6,463	<.0001	33% neg. tone	1 sec.
46	-42,47182	7,76	452	-5,475	0,0003	33% neg. tone	1 sec.
47	-9,66077	7,91	452	-1,221	1	33% neg. tone	1 sec.
48	-46,00293	7,76	452	-5,93	<.0001	33% neg. tone	1 sec.
49 - 84	No data					Out of set-up	
85 - 98	No data					break	

99 - 100	No data					Positive tone	1 sec.
101	-27,88783	8,65	452	-3,223	0,7449	10% neg. tone	1 sec.
102	-47,44199	8,37	452	-5,668	0,0001	10% neg. tone	1 sec.
103	-42,07783	8,14	452	-5,171	0,0013	20% neg. tone	1 sec.
104	No data					20% neg. tone	1 sec.
105	No data					50% neg. tone	1 sec.
106	-38,72392	8,14	452	-4,759	0,0087	50% neg. tone	1 sec.
107	-32,41572	8,14	452	-3,984	0,1529	50% neg. tone	1 sec.
108	-32,72479	8,14	452	-4,022	0,1362	50% neg. tone	1 sec.
109	-34,40073	8,14	452	-4,228	0,0694	50% neg. tone	1 sec.
110	-32,49595	8,14	452	-3,994	0,1484	50% neg. tone	1 sec.
111	-38,72392	8,14	452	-4,759	0,0087	50% neg. tone	1 sec.

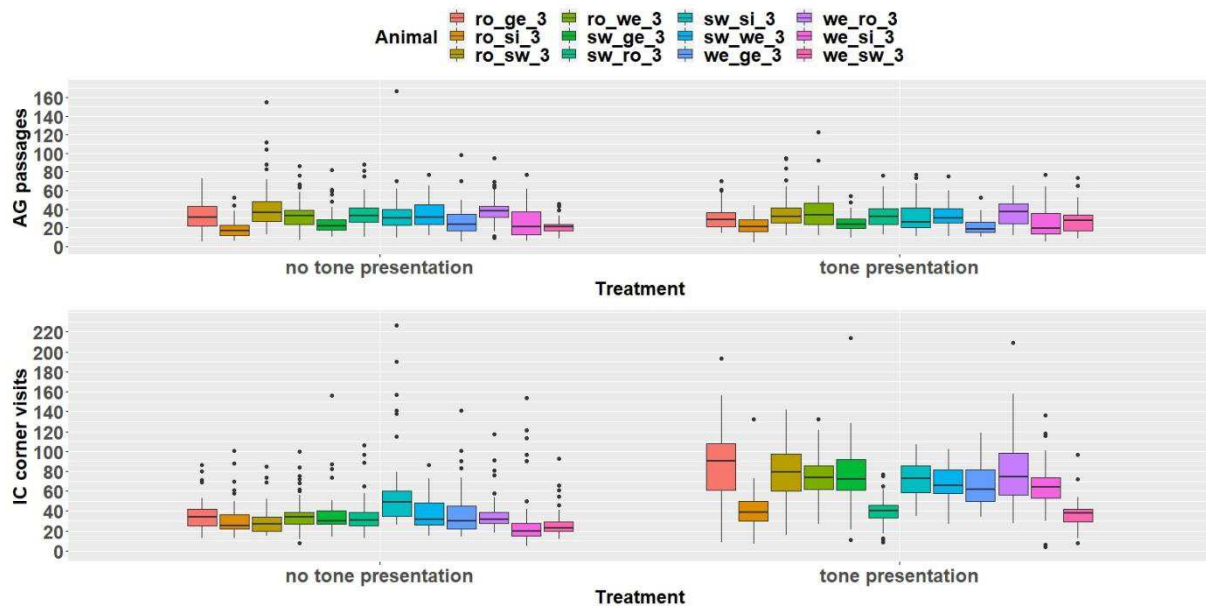
IntelliCage entries and corner visits

12 mice lived together as one social group in an automated and home-cage based set-up. Only one mouse were allowed to pass through the AnimalGate into the IntelliCage at a time. The IntelliCage corner can be visited multiple times per IntelliCage session. During the conditioning phase and/or the cognitive bias test, a tone (positive, negative or one of three ambiguous tones) was played at each visit (tone presentation). Depending on the experimental phase, there were periods when no tones were played (no tone presentation). Regardless of learning success, all 12 mice lived in this setup for the duration of the experiment. To determine whether all mice were able to enter the IntelliCage, the number of AnimalGate passes was first determined for each day and for each mouse. For ease of visualisation, a distinction was then made between days without tones presented and days with tones presented for each mouse. It was found that all mice were able to enter the IntelliCage repeatedly every day.

The number of corner visits appeared to vary between mice (for descriptive purposes only), as there were individual mice that did not make any corner visits even though they entered the IntelliCage several times. This suggests that individual mice seem to have an aversion to potential airpuffs (the risk of receiving an airpuff seems too high) and therefore did not visit the corner when they had the opportunity to do so.



IntelliCage entries group 2 developmental step 2: AG = AnimalGate, IC = IntelliCage, AnimalGate passages = IntelliCage entries = IntelliCage session, no tone presentation = time periods during which no tones were presented, tone presentation = time periods during which tones were presented. Shown are the IntelliCage sessions and corner visits per mouse for the phases when no tones were presented and when tones were presented. The mice ro_sw_2, sw_si_2, we_ro_2, we_si_2 reached the learning criterion.



IntelliCage entries group 3 developmental step 3: AG = AnimalGate, IC = IntelliCage, AnimalGate passages = IntelliCage entries = IntelliCage session, no tone presentation = time periods during which no tones were presented, tone presentation = time periods during which tones were presented. Shown are the IntelliCage sessions and corner visits per mouse for the phases when no tones were presented and when tones were presented. The mice ro_ge_3, ro_sw_3, ro_we_3, sw_ge_3, sw_si_3, sw_we_3, we_ge_3, we_ro_3 and we_si_3 reached the learning criterion.